

Figure 5 shows EAST inhibition curves of the Phl p 5b mutants using the allergic patient serum pool II/17.

E2

●—● nPhl p 5b	△—△ PM 1	◆—◆ DM 1	●—● DM 2
■—■ rPhl p 5b	▼—▼ PM 3	■—■ DM 3	⊖—⊖ DM 2*

Figure 6 shows release of histamine from human basophils after reaction with the allergens and allergen mutants.

✓✓  
Please delete pages 37-41 in their entirety.

✓  
On page 48, the first full paragraph has been deleted in its entirety.

✓  
On page 49, the first full paragraph has been amended as follows:

TABLE 7:

E3  
Proliferative reaction of Phl p 5b-reactive T cell clones (TCCs) from allergic patients with rPhl p 5b mutants

✓  
On page 50, the first full paragraph has been amended as follows:

TABLE 8:

E4  
Proliferative reaction of Phl p 5b-reactive T cell clones (TCCs) from allergic patients with rPhl p 5b mutants

On page 51, the first full paragraph has been amended as follows:

✓  
TABLE 9:

35 Proliferative reaction of PhI p 5b-reactive T cell clones (TCCs) from allergic patients

with rPhI p 5b mutants

On page 53, the first full paragraph has been amended as follows:

TABLE 10:

E6 Proliferative reaction of the PhI p 5b-reactive T cell lines (TCLs) from allergic patients

with rPhI p 5b mutants

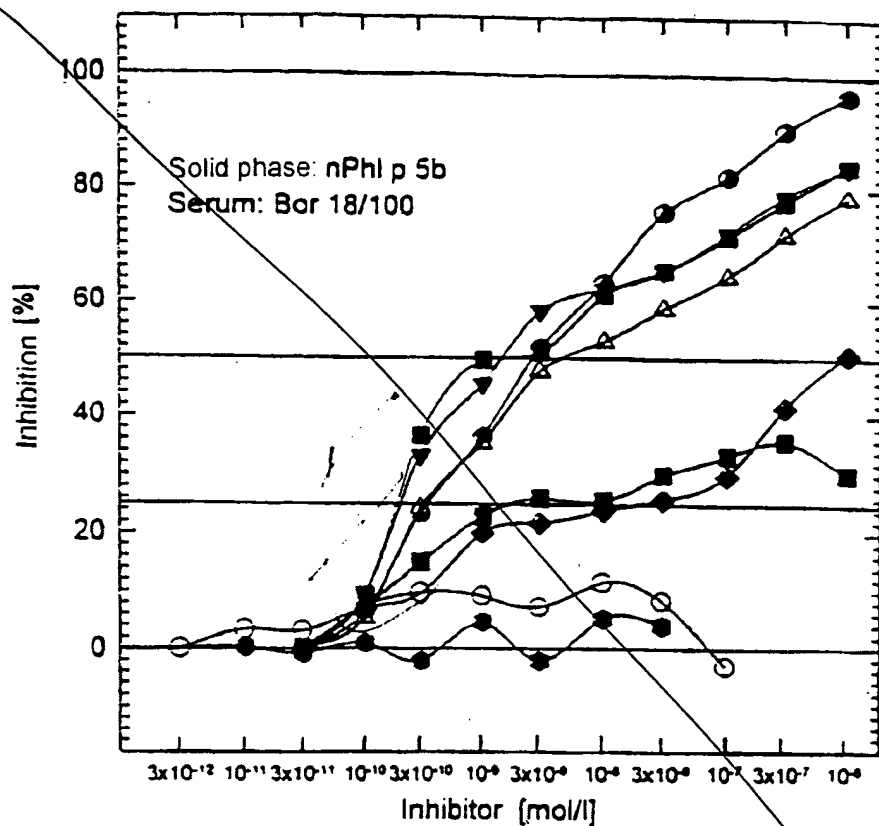


Figure 1 EAST inhibition curves of the Phl p 5b mutants using the allergic patient serum pool Bor 18/100

5 Inhibitors:

● nPhl p 5b    △ PM 1    ◆ DM 1    ● DM 2  
■ rPhl p 5b    ▼ PM 3    ■ DM 3    ○ DM 2\*

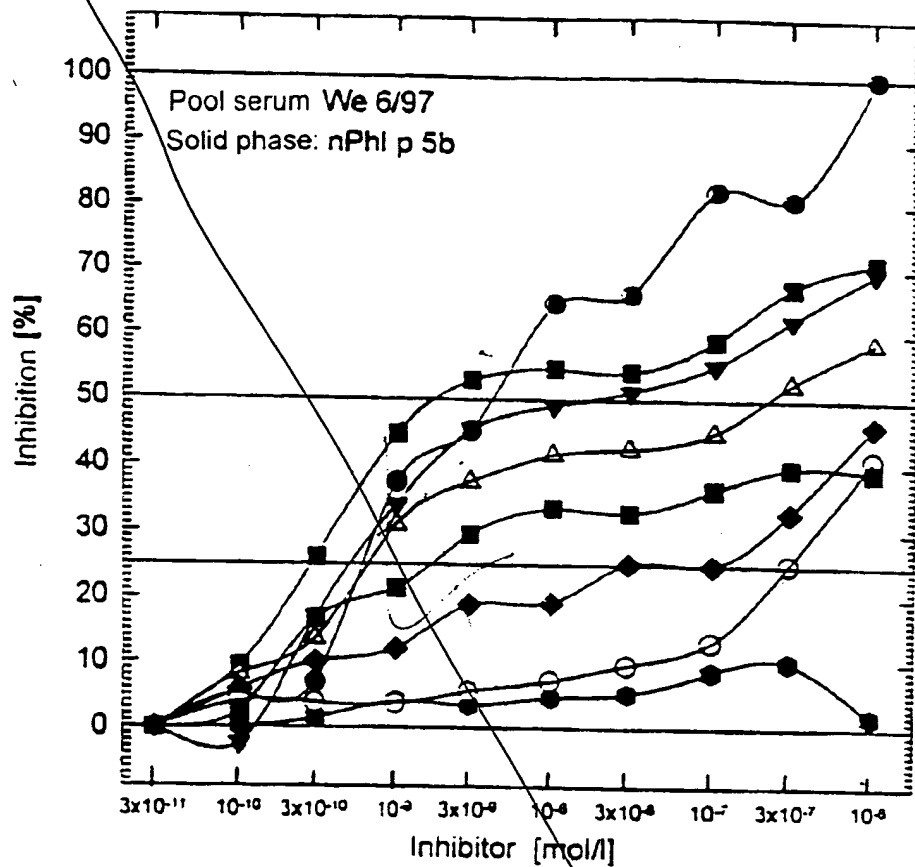
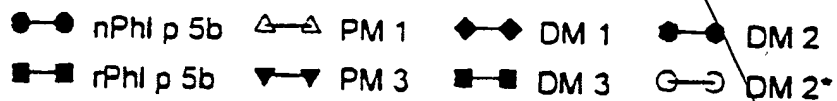


Figure 2 EAST inhibition curves of the Phl p 5b mutants using the allergic patient serum pool We 6/97

5 Inhibitors:



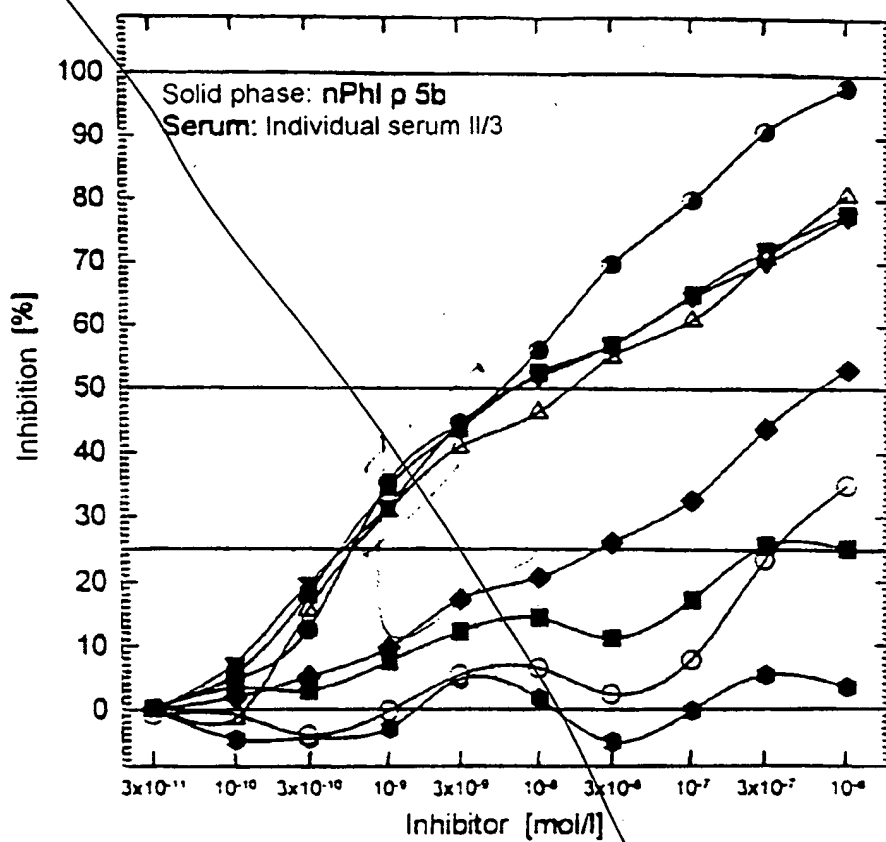
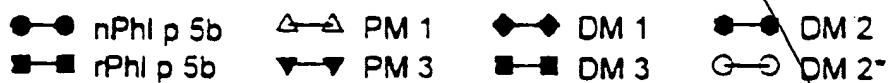


Figure 3 EAST inhibition curves of the Phl p 5b mutants using the individual allergic patient serum II/3

5 Inhibitors:



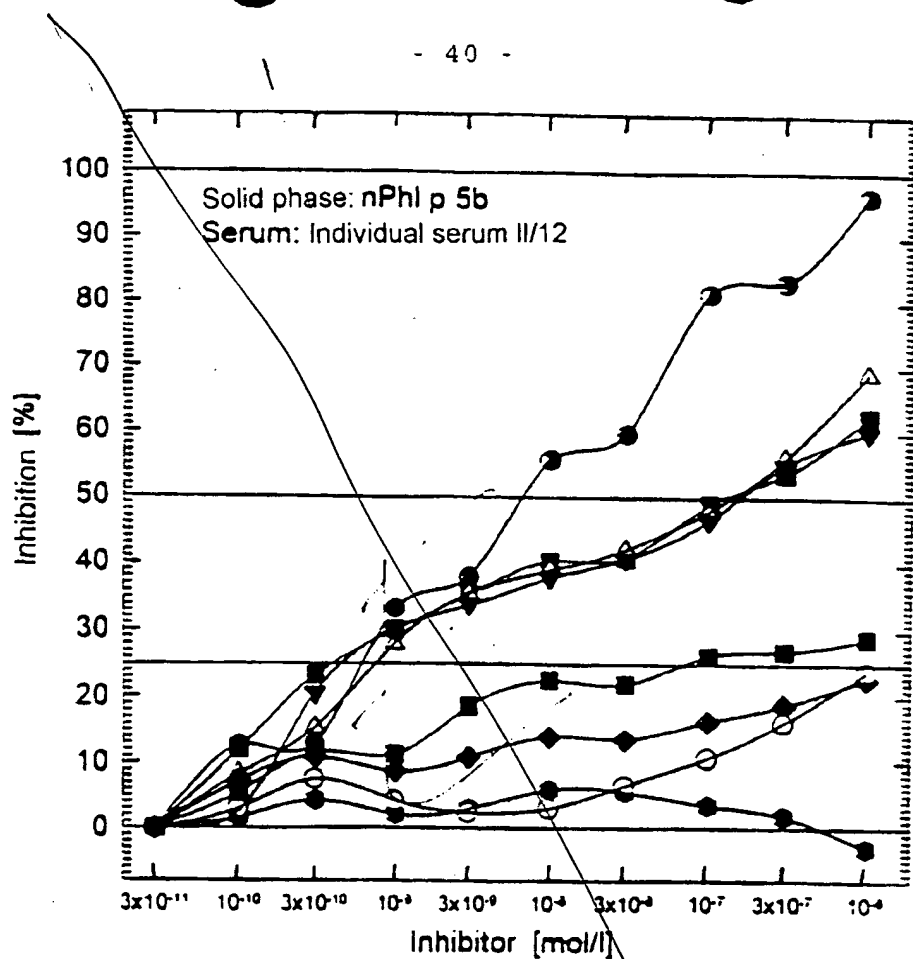
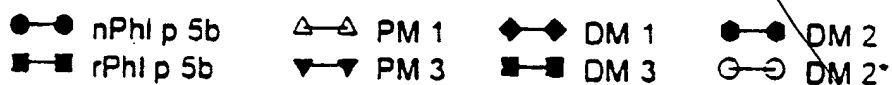


Figure 4 EAST inhibition curves of the Phl p 5b mutants using the individual allergic patient serum II/12

5 Inhibitors:



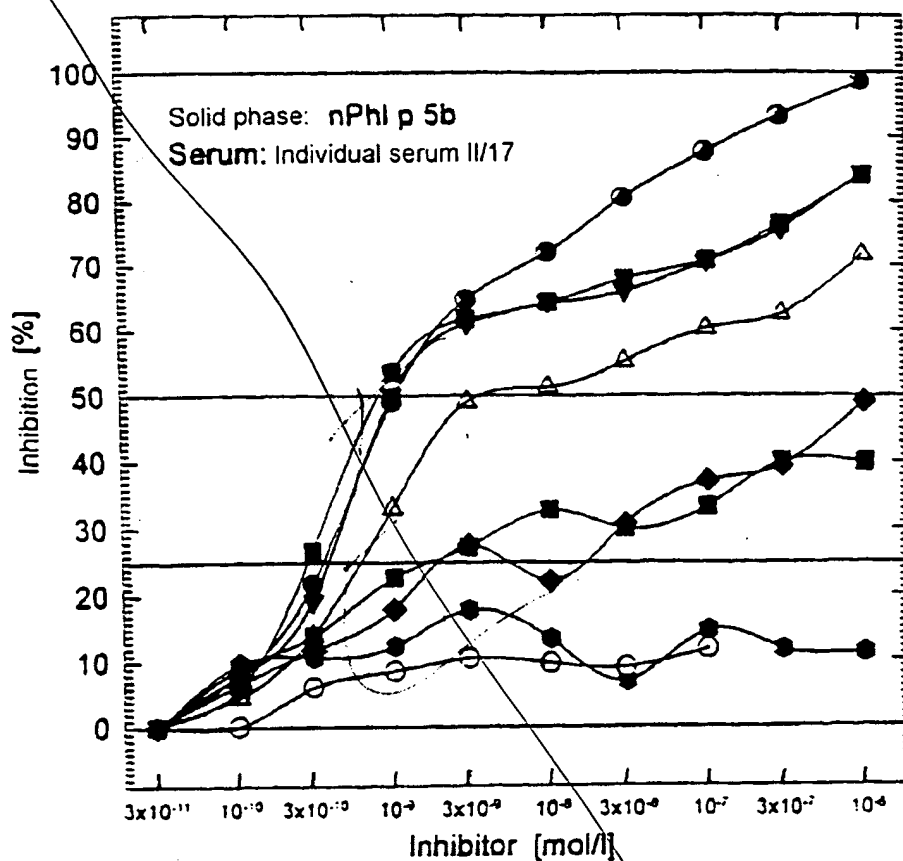


Figure 5 EAST inhibition curves of the Phl p 5b mutants using the individual allergic patient serum II/17

5 Inhibitors:

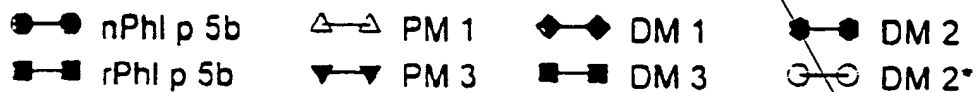
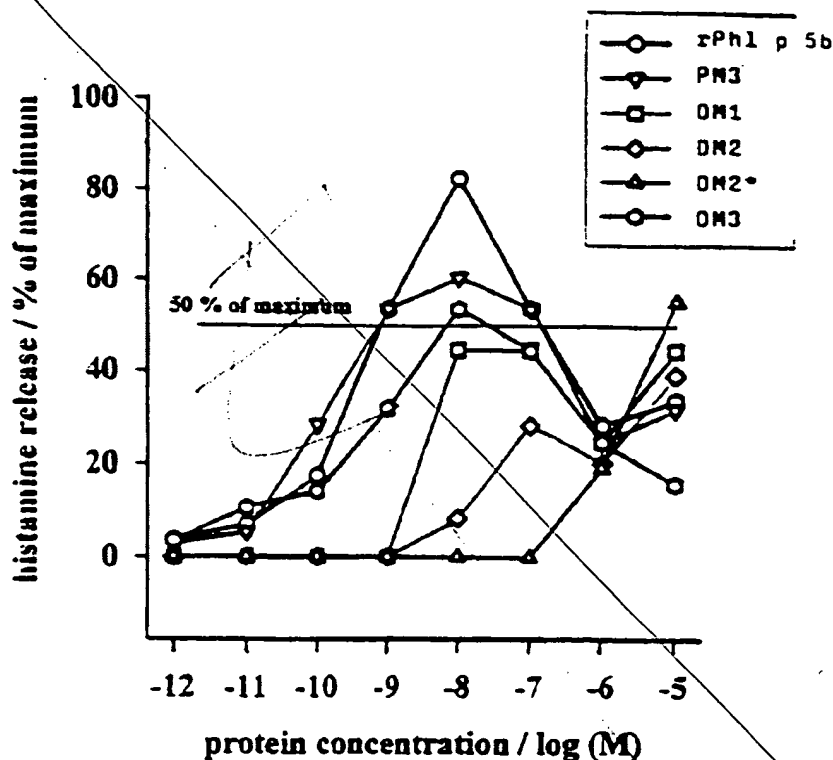


Fig. 6 Release of histamine from human basophils after reaction with the allergens and allergen mutants



# 5 Example 6

Demonstration of the reactivity of recombinant Phl p 5b mutants with T cell clones from patients who are allergic to grass pollen

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The reactivity of the recombinant Phl p 5b mutants was tested on established T cell clones (TCCs) of known specificity. The TCCs derive from patients who are allergic to grass pollen (see Ex. 1) and are directed against the T cell-reactive regions A (Fig. 7), B (Fig. 8) and C (Fig. 9). The T cell reactivity was measured by stimulating the clones to proliferate. The results clearly show that the TCCs react specifically with the Phl p 5b mutants when the relevant epitope is unaltered and, as expected, do not exhibit any reaction

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when the epitope is missing or is altered by a point mutation.

Fig. 7: Proliferative reaction of Phl p 5b-reactive T cell clones (TCCs) from allergic patients with rPhl p 5b mutants

Specificity: Immunodominant T cell-reactive region A

Stimulator <sup>1)</sup>	Epitope present	Incorporation of [ <sup>3</sup> H]-thymidine into TCC <sup>2)</sup>			
		JR 15	JR 13	CB 14	CB 2
Medium	-	-	-	-	-
n Phl p5	+	+++	+++	+++	+++
r Phl p5a	(±)	-	-	+	+
r Phl p5b	+	+++	+++	+++	+++
PM1	+	++	+++	+++	+++
PM3	+	+++	+++	+++	+++
DM1	+	+++	+++	+++	+++
DM2	+	+++	+++	nt <sup>3)</sup>	nt
DM2'	-	-	-	-	-
DM3	+	+++	+++	nt	nt
PL (12mer)	+	+++	+++	+++	+++

10 <sup>1</sup> Final concentration 0.3 µM

<sup>2</sup> Stimulation index SI: < 1 (-), 1-2 (+), 2-5 (+), 5-10 (++) , > 10 (+++)

<sup>3</sup> n.t.: not tested

Fig. 8: Proliferative reaction of Phl p 5b-reactive T cell clones (TCCs) from allergic patients with rPhl p 5b mutants

5 Specificity: Immunodominant T cell-reactive region B

Stimulator <sup>1)</sup>	Epitope present	Incorporation of [ <sup>3</sup> H]-thymidine into TCC <sup>2)</sup>	
		UZH2	DW8
Medium	-	-	-
n Phl p5	+	+++	+++
r Phl p5a	(±)	±	+++
r Phl p5b	+	+++	+
PM1	+	+++	+
PM3	+	+++	±
DM1	+	+++	+
DM2	-	-	-
DM2*	-	-	-
DM3	+	+++	+
PL (12mer)	+	+++	+++

<sup>1</sup> Final concentration 0.3 µM

<sup>2</sup> Stimulation index SI: < 1 (-), 1-2 (+), 2-5 (+), 5-10 (++), > 10 (+++)

<sup>3</sup> n.t.: not tested

Fig. 9: Proliferative reaction of Phl p 5b-reactive T cell clones (TCCs) from allergic patients with rPhl p 5b mutants

5 Specificity: Immunodominant T cell-reactive region C

Stimulator <sup>1)</sup>	Epitope present unchanged	Incorporation of [ <sup>3</sup> H]-thymidine <sup>2)</sup>			
		1 <sup>st</sup> exp.	2 <sup>nd</sup> exp.	3 <sup>rd</sup> exp.	
Medium	-	1	1	1	-
n Phl p5	+	11.2	8.2	4.5	++
r Phl p5a	-	nt	<1	<1	-
r Phl p5b	+	11.0	7.0	5.5	++
PM1	-	<1	<1	1.1	-
PM3	+	7.4	5.9	4.5	++
DM1	+	8.6	6.2	4.4	++
DM2	+	14.4	9.1	7.1	+++
DM2 <sup>*</sup>	+	12.8	12.1	11.7	+++
DM3	+	9.8	6.9	4.4	++
PL (12mer)	+	20.9	15.7	nt <sup>3)</sup>	+++

<sup>1)</sup> Final concentration 0.3  $\mu$ M

<sup>2)</sup> Stimulation index SI: < 1 (-), 1-2 (+), 2-5 (+),

10 5-10 (++) , > 10 (+++)

<sup>3)</sup> n.t.: not tested

